

# Patterns in Early Embryonic Motility: Effects of Size and Environmental Temperature on Vertical Velocities of Sinking and Swimming Echinoid Blastulae

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**Abstract.** Early embryonic swimming is widespread among marine invertebrates, but quantitative information about swimming behaviors is scarce. Swimming may affect encounters with predators, positioning in the water column, and nutrient absorption. Measured rates and patterns of swimming and sinking for blastulae of four eastern Pacific echinoid species show that sinking speeds equal or exceed swimming speeds. Swimming speed scaled negatively with embryo size, though sinking speed did not scale with size. Analysis of swimming paths of *Strongylocentrotus franciscanus* revealed a temperature dependency in swimming pattern that affected speed of upward movement. Sinking speeds were significantly greater at 10 °C than at 14 °C for blastulae of all four species examined. In *Dendraster excentricus*, killing the blastulae annulled this temperature effect, indicating an active density regulation by these embryos. Finally, measurements of particle velocities around sinking and swimming *D. excentricus* blastulae show that swimming creates a more localized disturbance than sinking. Embryonic swimming may therefore decrease rather than increase encounters with pelagic predators. Results from subsequent experiments in which embryos were reared in low-oxygen environments suggest that any oxygen-absorption advantages of swimming have little, if any, effect on the development of *D. excentricus* embryos.

## Introduction

The embryos of some benthic marine invertebrates develop singly in the plankton; others develop in benthic masses. While modes of embryogenesis vary, planktonic

embryos of diverse taxa demonstrate early swimming ability. Solitary embryos of many species begin swimming hours prior to gastrulation. Embryos encased in egg masses may begin rotating within their capsules long before hatching. While even closely related species may differ in the timing of first swimming, positive correlations between time to first swimming and factors including egg size and time to first cleavage can account for much of the variability observed in age or stage at first swimming (Staver and Strathmann, 2002).

The ubiquity of early swimming among pelagic invertebrate embryos raises questions about its evolution and ecological consequences. The functional morphology of many invertebrate larval forms has been extensively explored; embryos are worthy of similar scrutiny, given that the survivorship of early stages has direct consequences for the viability of any developmental strategy. Interactions with predators, positioning in the water column, and uptake of dissolved nutrients are a few of the areas in which swimming could affect embryonic survival.

It has occasionally been demonstrated, and more frequently assumed, that larvae are more vulnerable to predation at early developmental stages in consequence of their smaller size and limited maneuverability (Pennington and Chia, 1984; Rumrill *et al.*, 1985; Pennington *et al.*, 1986; Rumrill, 1990). Non-swimming embryos might be still more vulnerable. In theory, flow-field differences between swimming and sinking particles produce different signals in the water column; ciliary swimming creates a steeper velocity gradient and therefore a more localized disturbance (Vogel, 1994). Sinking embryos might be more readily detectable by ambush predators. Conversely, swimming could increase encounter rates between predators and prey in a given volume of water (Gerritsen and Strickler, 1977;

Kjørboe and Saiz, 1995). Studies of interactions between planktonic predators and their prey (largely with copepods) have examined prey organisms with escape responses to predator proximity (Yen, 1988; Yen and Strickler, 1996; Kjørboe *et al.*, 1999); early embryos possess no such elaborate behaviors. But because the swimming capabilities of larvae play a large role in their ability to evade capture, we can also speculate that predators of embryos might experience more difficulty in handling swimming rather than non-swimming embryos.

A second hypothesis is that swimming enables a vertical migration into the water-column. Laboratory studies in still water indicate that most solitary embryos swim upward. Benthic development is associated with greater parental investment in embryos, in the form of gel layers, extra-embryonic capsules, and parental care (Pechenik, 1979; Strathmann, 1985; Lee and Strathmann, 1998; Bolton *et al.*, 2000). The perils implied by such protection suggest one advantage of vertical migration by solitary embryos. Still, the effectiveness of upward swimming must depend in large part upon the turbulence regimes embryos encounter (Metaxas, 2001). There is no cause to assume that the net upward movement of pelagic early swimming stages differs from that of pre-swimming stages in a mixed water column.

Developing sea urchin embryos consume oxygen at a near-constant rate during the early blastula stage (Yanagisawa, 1975; Isono and Yasumasu, 1968). At particle sizes characteristic of many invertebrate embryos (diameter 50–250  $\mu\text{m}$ ), oxygen absorption could be significantly increased through the changes in near-field flow brought about by swimming, as compared with passive sinking (Berg and Purcell, 1977). One hypothesis is that swimming enhances uptake of oxygen or other dissolved nutrients sufficiently to accelerate embryonic growth, thus increasing survivorship of embryos. Oxygen limitations for non-swimming embryos could impose a selective pressure on the timing of swimming in marine invertebrate embryogenesis.

Few published data are available on embryonic swimming abilities and behaviors. To explore the potential significance of early swimming to embryonic survival, it is necessary to know how physical characteristics of embryos (such as size and ciliation pattern) affect swimming, and how responsive swimming behaviors are to changes in environmental conditions. Even well-mixed water columns have some temperature and salinity structure, which could affect embryo distributions by altering swimming performance. Effects of swimming on nutrient or dissolved-gas uptake and on predator encounter rate are likely to depend upon speeds and patterns of embryonic swimming (Berg and Purcell, 1977; Rubinstein and Koehl, 1977; Shimeta and Jumars, 1991; Kjørboe and Saiz, 1995).

In a laboratory study, I have quantified sinking and swimming speeds of early blastulae from four echinoid species whose similar early development makes them a natural

experimental system for investigating swimming behavior. Embryos of the sand dollar *Dendraster excentricus* (Eschscholtz, 1831) and the urchins *Strongylocentrotus droebachiensis* (Mueller, 1776), *S. franciscanus* (Agassiz, 1863), and *S. purpuratus* (Stimpson, 1857) develop to the blastula stage enclosed in a fertilization envelope. These species form coeloblastulae composed of monociliated cells. Blastulae begin rotating within the envelope prior to hatching, and upward swimming commences shortly after hatching. Upward orientation is passive and defined by the animal-vegetal axis. Although all embryos rotate as they swim, the angle of inclination from the vertical along the A-V axis varies within and among species. Under the conditions of this study, the blastulae of *D. excentricus* and *S. purpuratus* demonstrated little to no inclination of the A-V axis, whereas those of *S. droebachiensis* and *S. franciscanus* demonstrated a more noticeable inclination.

The objectives were to determine how swimming and sinking scale with size, if at all; how sinking speeds compare with swimming speeds; and whether these rates of movement are sensitive to changes in environmental temperature. In each species, recently hatched blastulae were the swimmers and unhatched blastulae the sinking embryos. The comparisons made are therefore akin to comparisons between swimming embryos and embryos in which hatching has been deferred to a later stage, rather than embryos that never develop ciliation.

Two extensions of this study are also presented. To test the hypothesis that swimming produces a more restricted signal to predators, I quantified flow characteristics around sinking (pre-hatching) and swimming embryos of *D. excentricus*. To evaluate the hypothesis that swimming rescues embryos from oxygen-limited conditions, the negative effects of lowered oxygen on embryogenesis must be demonstrated. Swimming could provide embryos with a diffusive advantage that they never need. For this reason, I examined the effects of decreased ambient oxygen on the growth and development of *D. excentricus* embryos.

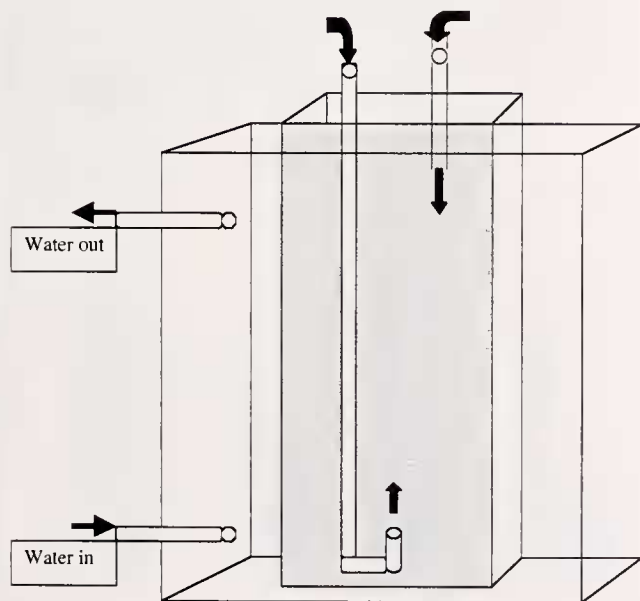
## Materials and Methods

Adult urchins (*Strongylocentrotus droebachiensis*, *S. purpuratus*, and *S. franciscanus*) and sand dollars (*Dendraster excentricus*) were collected and subsequently held at the Friday Harbor Laboratories, San Juan Island, Washington. Spawning was induced in ripe adults by injection of 0.53 M KCl. Embryos were reared on sea tables at temperatures of about 9.5°–12 °C until they were used in experiments. One to two hours prior to hatching, "sinking" (pre-hatching) embryos were taken from culture dishes and placed in syringes. If still present, jelly coats were removed by filtering embryos through Nitex mesh. Similarly, swimming embryos were taken from culture dishes between 1 and 2 h after hatching. Syringes were then incubated in

water baths for 10–15 min so that embryos could acclimate to the temperature within each experimental chamber.

Each chamber used to measure sinking and swimming rates consisted of a rectangular glass box nested within a rectangular plastic jacket (Fig. 1). Water from a temperature-controlled bath was recirculated through the jacket to bring the temperature within the inner chamber to either 10 °C or 14 °C. The inner chamber (outside dimensions: 12 cm high × 5.2 cm wide × 2.5 cm deep) was large enough to preclude wall effects in the central 0.8 cm of the box's depth. (The extent of wall effects on moving embryos was separately determined; see Discussion and Fig. 9.) A Sony video camera with a C-mounted macro lens (Canon, 55 mm/f:2.8) was focused on the center of the chamber and recorded the paths of embryos moving through its field of view. Sinking embryos were introduced into the top of the inner chamber *via* a standing glass tube; as they sank through the tube, they separated and then fell individually at terminal velocity past the camera. Swimming embryos were introduced *via* an L-shaped glass tube resting on the bottom of the chamber. A scale attached to the back of the inner chamber allowed for calibration of the recorded images.

After recordings were completed, vertical swimming speeds (and in some instances, swimming patterns) were quantified for individual embryos. Only isolated embryos swimming or falling past the camera were used in these measurements. Recordings at 10 °C and 14 °C were made simultaneously, with parallel apparatus and the same culture of embryos.



**Figure 1.** Diagram of experimental chamber used to measure rates of embryonic sinking and swimming. The chamber nested within a recirculating water bath that maintained seawater in the chamber at 10 °C or 14 °C. Blastulae were introduced to the chamber *via* syringe and glass tube.

To quantify flow-field characteristics of sinking and swimming *D. excentricus* embryos, a small chamber was built to fit on the stage of a horizontally mounted microscope. The chamber consisted of a 2-cm plastic petri dish, glued closed and inserted into a small acrylic plastic frame cut to size. Two small holes were drilled into one face of the dish. The circular chamber was filled with seawater and a suspension of 4.7- $\mu$ m polystyrene divinylbenzene beads (Duke Scientific). The chamber was mounted on the microscope stage, and individual embryos were then gently introduced to its interior *via* a "slide" made of plastic tubing and one of the holes. Embryos moving in the bead suspension were tracked and video-recorded as they sank or swam, precluding repeated recordings of any individual. Video clips of bead movement around sinking and swimming embryos were then analyzed using ImageJ software (ver. 1.32 for Macintosh; available at <http://rsb.info.nih.gov/ij/>). Bead velocity could be determined frame by frame, yielding velocity profiles around sinking and swimming embryos.

*Dendraster* embryos were reared in three different oxygen environments to determine the sensitivity of embryonic growth and development to oxygen deprivation. Nitrogen gas was bubbled into two small tanks immersed in the same table of running seawater; rates of bubbling were adjusted to achieve stable (over a 36–48-h period) oxygen saturations of 6%–10% in the lowest oxygen treatment and 20%–25% in the intermediate oxygen treatment. Air bubbled into a third tank produced an oxygen saturation of 63%–69%. Embryos from a single culture were introduced to a mesh-sided container in each tank within an hour after insemination. Treatment containers were not mechanically stirred. A digital oxygen meter enabled frequent monitoring of oxygen levels within containers. At nine intervals throughout early development (16–45 h at 12°–13 °C; pre-hatching until prism stage), embryos were removed from each treatment and their images captured with a video microscope for later analysis. Data obtained for embryos included three measures of size as well as stage information (*e.g.*, whether embryos were rotating or nonrotating, hatched or unhatched), and abnormality rates.

## Results

Blastula sizes for the four species examined are listed in Table 1. Post hoc Tukey HSD tests for between-species comparisons of sinking and swimming are summarized in Table 2.

Embryonic sinking speeds did not scale with size. At 10 °C, the smallest embryos (*Strongylocentrotus purpuratus*) sank significantly faster than the largest (*S. droebachiensis*), and at 14 °C, embryos of the three strongylocentrotid species sank at statistically indistinguishable rates (Table 2). These results indicate density differences among embryos of the four species examined. *S. purpuratus* embryos had

Table 1

Embryo size and calculated density

| Species                              | Mean unhatched blastula diameter<br>( $\mu\text{m}$ ) with fertilization envelope<br>( $n = 10$ ) | Calculated density ( $\text{kg m}^{-3}$ )* |
|--------------------------------------|---|--|
| <i>Dendraster excentricus</i>        | 159.8   | $1.063 \times 10^3 \pm 0.007 \times 10^3$  |
| <i>Strongylocentrotus purpuratus</i> | 104.9   | $1.120 \times 10^3 \pm 0.023 \times 10^3$  |
| <i>S. franciscanus</i>               | 170.2   | $1.072 \times 10^3 \pm 0.012 \times 10^3$  |
| <i>S. droebachiensis</i>             | 211.7   | $1.049 \times 10^3 \pm 0.003 \times 10^3$  |

\* Calculations made using mean sinking rates of embryos at 10 °C; for seawater at 10 °C,  $\mu = 1.391 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$ ,  $\rho = 1.024 \times 10^3 \text{ kg m}^{-3}$  at salinity of 30 ppt; note that unhatched embryos of these 4 species fill their fertilization envelopes to similar extents (65.3%–68.8% of total volume).

the greatest calculated density, followed by *S. franciscanus*, *Dendraster excentricus*, and *S. droebachiensis* (Table 1).

There was a strong negative correlation between swimming rate and embryo size (Fig. 2). This was true at both temperatures, so graphical data from only the 10 °C trials are shown. Sinking rates were significantly greater than swimming rates for three of the four species examined (Student's *t* tests,  $P < 0.001$  in each case); the exception was *S. purpuratus*, whose embryos sank and swam at statistically indistinguishable rates (*t* test,  $P = 0.45$ ) (Fig. 3). Again, this relationship holds true at both temperatures.

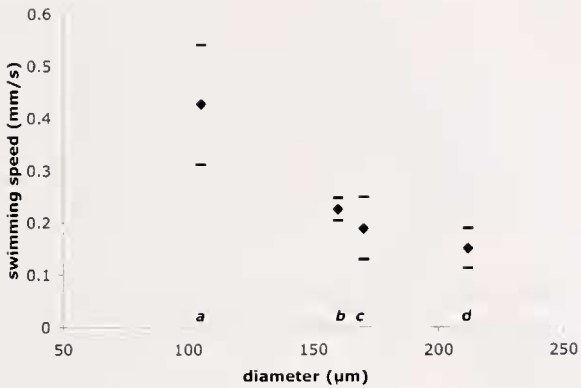
Effects of temperature on embryonic swimming rate varied by species, with one species (*D. excentricus*) swimming significantly more rapidly at 14 °C, and another (*S. franciscanus*) swimming more rapidly at 10 °C (*t* tests:  $P < 0.01$ ,  $P = 0.05$  respectively) (Fig. 4A). At 14 °C, *S. droebachiensis* embryos could seldom swim upward for more than a few millimeters at one time, resulting in a small sample size. Embryonic sinking rates were also sensitive to temperature. Unhatched blastulae of each species sank significantly more rapidly at 10 °C than at 14 °C (*t* tests:  $P \leq 0.01$  for each comparison) (Fig. 4B). When unhatched *D. excentricus* blastulae were killed with 3  $\mu\text{M}$  2,4-dinitrophe-

Table 2

Between-species comparisons of sinking and swimming rates at 10° and 14 °C for *Strongylocentrotus franciscanus*, *S. purpuratus*, *S. droebachiensis*, and *Dendraster excentricus*

| 10°                  | Fastest                |                       |                          | Slowest                  |
|----------------------|------------------------|-----------------------|--------------------------|--------------------------|
| <b>Sinking</b>       |                        |                       |                          |                          |
| Species              | <i>S. franciscanus</i> | <i>S. purpuratus</i>  | <i>S. droebachiensis</i> | <i>D. excentricus</i>    |
| <i>n</i>             | 31                     | 29                    | 38                       | 70                       |
| Mean (mm/s) $\pm$ SD | $0.522 \pm 0.1315$     | $0.448 \pm 0.1057$    | $0.390 \pm 0.0449$       | $0.365 \pm 0.0702$       |
| <b>Swimming</b>      |                        |                       |                          |                          |
| Species              | <i>S. purpuratus</i>   | <i>D. excentricus</i> | <i>S. franciscanus</i>   | <i>S. droebachiensis</i> |
| <i>n</i>             | 30                     | 80                    | 50                       | 32                       |
| Mean (mm/s) $\pm$ SD | $0.426 \pm 0.1145$     | $0.218 \pm 0.0160$    | $0.189 \pm 0.0597$       | $0.150 \pm 0.0383$       |
| 14°                  | Fastest                |                       |                          | Slowest                  |
| <b>Sinking</b>       |                        |                       |                          |                          |
| Species              | <i>S. franciscanus</i> | <i>S. purpuratus</i>  | <i>S. droebachiensis</i> | <i>D. excentricus</i>    |
| <i>n</i>             | 39                     | 32                    | 53                       | 78                       |
| Mean (mm/s) $\pm$ SD | $0.416 \pm 0.0690$     | $0.384 \pm 0.0861$    | $0.369 \pm 0.0260$       | $0.307 \pm 0.1080$       |
| <b>Swimming</b>      |                        |                       |                          |                          |
| Species              | <i>S. purpuratus</i>   | <i>D. excentricus</i> | <i>S. franciscanus</i>   | <i>S. droebachiensis</i> |
| <i>n</i>             | 37                     | 53                    | 56                       | 19                       |
| Mean (mm/s) $\pm$ SD | $0.411 \pm 0.1065$     | $0.264 \pm 0.0641$    | $0.158 \pm 0.0820$       | $0.133 \pm 0.0335$       |

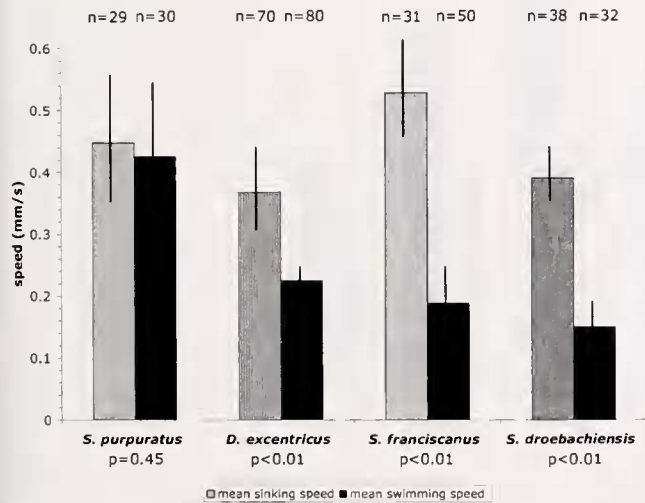
Results of Tukey HSD test after ANOVA (SYSTAT program).  $H_0$ : no effect of species on rate of embryo movement (swimming or sinking). All ANOVA tests rejected  $H_0$  at  $P < 0.001$ : (10°, sinking:  $F = 26.474$ ; 10°, swimming:  $F = 141.229$ ; 14°, sinking:  $F = 19.414$ ; 14°, swimming:  $F = 81.476$ ).



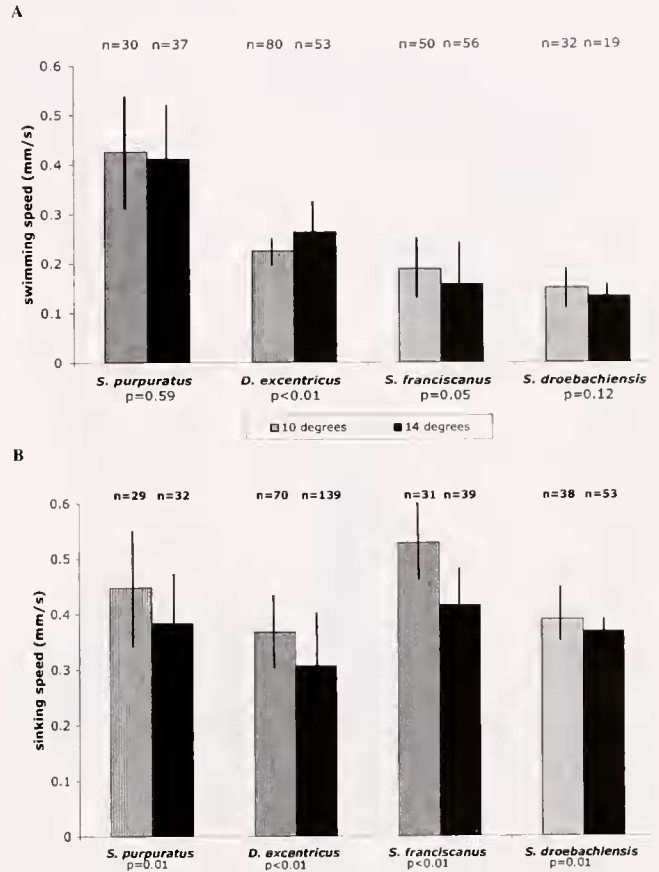
**Figure 2.** Mean swimming rates for blastulae at 10 °C. Bars show one standard deviation about the mean. **a** = *Strongylocentrotus purpuratus* (n = 30), **b** = *Dendraster excentricus* (n = 80), **c** = *S. franciscanus* (n = 50), **d** = *S. droebachiensis* (n = 32)

nol. a metabolic inhibitor (Flickinger, 1972), this temperature effect was negated.

A closer examination of the swimming pattern of *S. franciscanus* at the two temperatures revealed that the decrease in swimming rate at 14 °C was proximally caused by a change in swimming pattern. The radius of the helical path followed by swimming embryos was most frequently 1 embryo diameter or less, making accurate measurement of radii difficult, but helical period could vary widely among individuals. For ease of data extraction, I measured the period length (Fig. 5A). As temperature increased, the distribution of period frequencies shifted (Kolmogorov-Smirnov test for discrete data, significant difference at  $\alpha < 0.001$ ) such that, on average, embryos at 14 °C were following paths with greater period (Fig. 5B). This had the effect of increasing the distance traveled between two vertical points, slowing their rate of ascent.



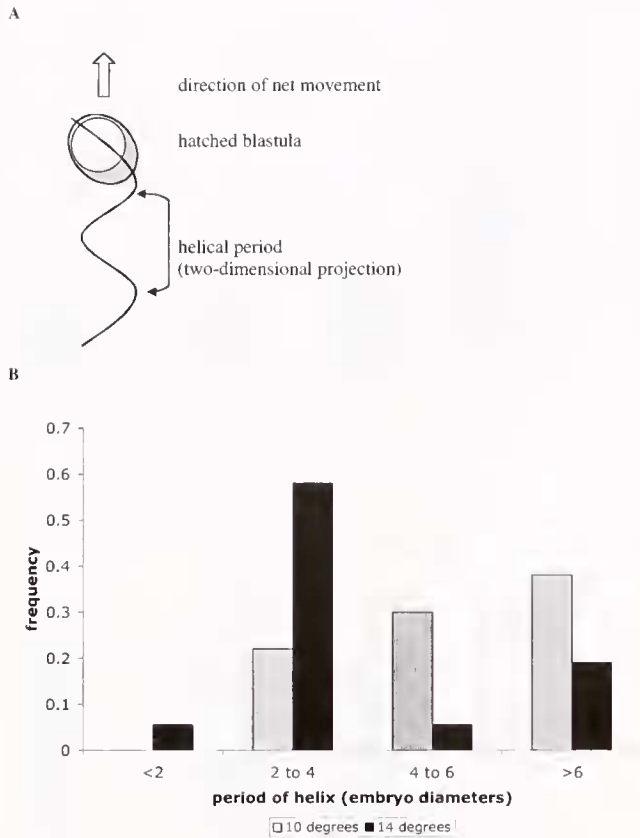
**Figure 3.** Comparison of sinking rates with swimming rates at 10 °C. P values for two-tailed t tests shown for each within-species comparison.



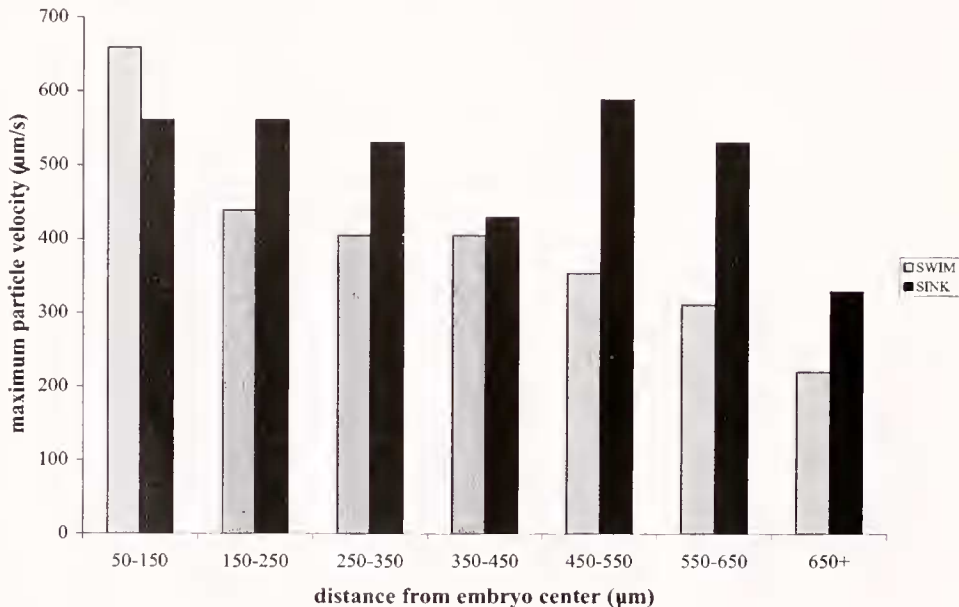
**Figure 4.** Sinking and swimming rates at two temperatures: light stippling indicates 10 °C; dark stippling indicates 14 °C. **(A)** Comparison of swimming rates at 10 °C and 14 °C. P values for two-tailed t tests shown for each within-species comparison. **(B)** Comparison of sinking rates at 10 °C and 14 °C. P values for two-tailed t tests shown for each within-species comparison.

High particle velocities around sinking and swimming *D. excentricus* embryos are shown in Figure 6. Swimming embryos produced a steep velocity gradient: maximum particle speed diminished rapidly with distance from the embryo surface. Sinking embryos produced a gentler velocity gradient that disturbed the water column at a greater distance from the moving embryo.

Oxygen deprivation had few significant effects on embryo development when embryos were reared in low-oxygen environments from the time of fertilization. A randomized blocked ANOVA (SYSTAT 9.0, Systat Software Inc.) showed significant effects of sample time on embryo length parallel to the animal-vegetal axis ( $df = 4, F = 3.515, P = 0.013$ ), but no effects of oxygen treatment ( $df = 2, F = 0.612, P = 0.546$ ) (Fig. 7A). The same ANOVA model showed significant effects of oxygen treatment on length perpendicular to the animal-vegetal axis ( $df = 2, F = 3.863, P = 0.024$ ; Fig. 7B), but the spread of mean values (aver-



**Figure 5.** Illustration of variability in upward helical swimming paths of *Strongylocentrotus franciscanus* blastulae. (A) Two-dimensional schematic projection of the helical swimming pattern. (B) Distribution of helical periods among swimming blastulae at 10 °C and 14 °C. Kolmogorov-Smirnov test for discrete data, significant at  $\alpha < 0.001$ .



**Figure 6.** Maximum particle velocity around sinking and swimming *Dendraster excentricus* embryos as a function of distance from the embryo center. Outliers (defined as particles in each distance bin moving  $\geq 50$   $\mu\text{m/s}$  faster than the next fastest particle) were removed.

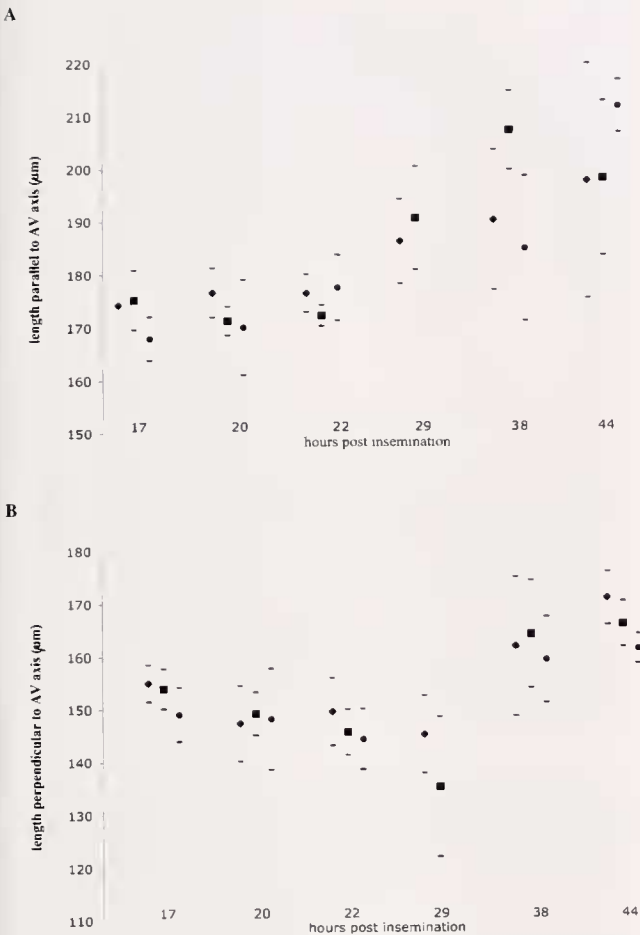
aged over all time segments for each treatment) encompassed a range of only 5  $\mu\text{m}$ .

Some aspects of developmental timing demonstrated oxygen sensitivity. The frequency with which embryos were seen to rotate during the first three sampling times (prior to hatching) varied significantly by oxygen treatment, with sensitivity beginning at the intermediate oxygen level, or 20%–25% saturation (Fig. 8). The timing of vegetal plate buckling was accelerated by 2–3 h in the high oxygen treatment, as compared with the intermediate oxygen treatment (chi-square test,  $P < 0.05$ ). At the sampling time 38 h post-fertilization, the three treatments varied significantly in the distribution of stages present: high-oxygen embryos were all prisms, while some low-oxygen embryos had not yet begun gastrulating. At this particular sampling time, each oxygen treatment showed a statistically distinct distribution of developmental stages (chi-square tests,  $P < 0.001$  for each pairwise comparison).

## Discussion

### *Embryo size and swimming speed*

Stokes' law states that drag increases linearly with spheroid radius and speed of movement when Reynolds numbers are below unity. Terminal velocity at low Reynolds numbers, however, scales with the square of radius, since volume (and therefore mass) grows more rapidly than drag with increasing radius. Reynolds numbers calculated for the blastulae in this study were in the range of 0.02–0.06. If embryos are of equal density, their terminal velocities should scale positively with embryo size. This was not the



**Figure 7.** Expansion of *Dendraster excentricus* embryos in three oxygen-depleted environments. (A) Expansion along the animal-vegetal axis. (B) Expansion perpendicular to the animal-vegetal axis. Solid shapes indicate mean lengths in  $\mu\text{m}$  with corresponding SD bars. Solid diamond = high oxygen treatment (63%–69% saturation); solid square = intermediate oxygen treatment (20%–25% saturation); solid circle = low oxygen treatment (5%–9% saturation). One sampling interval (29 h post insem.) was missed for the low oxygen treatment. Note that sampling intervals are arranged categorically, not to scale.

case for embryos of the four species examined here, which indicates differences in interspecific density. Calculated densities are reported in Table 1.

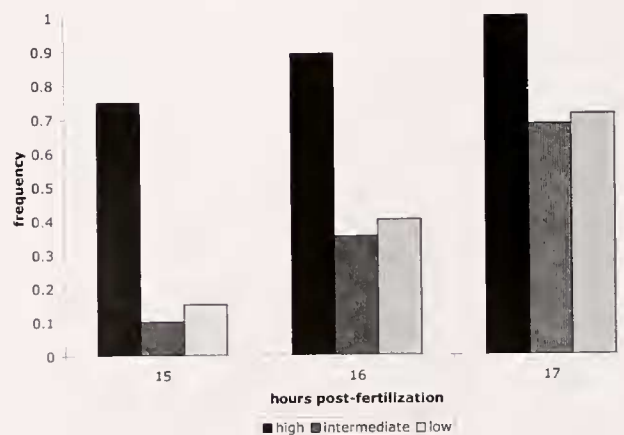
While forces of drag and thrust acting on ciliated swimming embryos cannot be simply modeled using Stokes' law, some of the same relationships can guide our thinking about the factors influencing swimming speed. If, for instance, embryos of these species had about the same density and similar thrust-generating capacity—if they had roughly the same number of cells and hence cilia at hatching—then the strong negative scaling of speed with size could be due mainly to drag effects. However, there is evidence that smaller embryos can achieve greater upward velocity than their larger congeners with less investment in thrust. Kohtaro Tanaka (University of Washington; pers. comm.)

found that *Dendraster excentricus* blastulae have 1200–1500 cells at hatching, while Hinegardner (1967) found approximately 350 cells for *Strongylocentrotus purpuratus*.

In other discussions of the tradeoff between embryo size and survivorship in invertebrates, small egg size is usually considered a liability for planktonic embryos. Small size may increase the number of predators capable of ingesting an embryo (Hansen *et al.*, 1994). Also, small planktotrophic embryos may need to spend more time feeding before they are capable of metamorphosis, extending their period of high mortality risk in the plankton (Vance, 1973; Strathmann, 1985; Sinervo and McEdward, 1988; Rumrill, 1990), though size-advantage hypotheses for early embryos are not strongly supported by data. The observed relationship between swimming speed and embryo size suggests one possible advantage of small egg size from the embryo's perspective. Since only four closely related species were used in establishing this relationship, a wider sampling of embryonic swimming rates would be useful.

#### Effects of temperature on motility

The increased sinking rates of pre-hatching blastulae at 10 °C as compared with 14 °C had implications for embryonic density. Because the viscosity of seawater increases with decreasing temperature, I expected to observe a slight increase in sinking rate with the 4 °C increase in temperature. The uniformity of the result (significant for all four species) suggested a previously unsuspected active density regulation by these unhatched blastulae. Because killed embryos at both temperatures reverted to sinking rates statistically similar to those of living embryos at 14 °C, it seems that the regulatory mechanism is acting at the lower



**Figure 8.** Frequency of occurrence of rotation in unhatched embryos reared in three oxygen environments. Embryos were scored as rotating or non-rotating. Chi-square tests distinguish between the high oxygen treatment as compared with low and intermediate treatments ( $P < 0.01$  for each pairwise comparison), whereas the low and intermediate treatments are indistinguishable at any time point ( $P > 0.05$ ).

rather than the higher temperature. It was expected that a metabolic inhibitor (2,4-dinitrophenol) would kill embryos without affecting their permeability, and the sinking speeds measured for killed embryos appear to support this assumption.

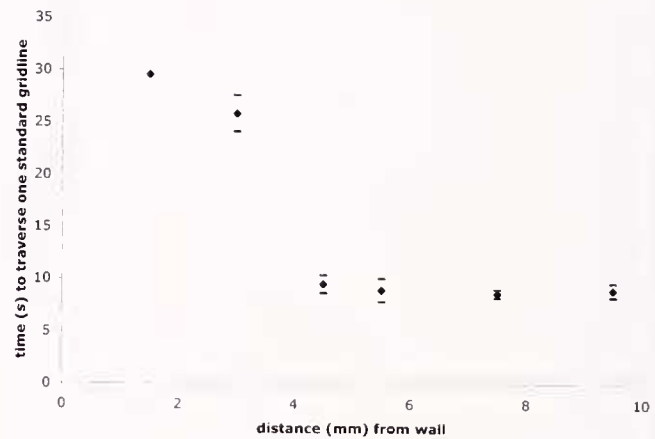
Tracking the sinking speeds of *D. excentricus* blastulae during development indicates that density regulation is occurring as early as the 7th cleavage, when the blastocoel begins to take shape (about the same time intercellular junctions are forming; Okazaki, 1975). Further investigation is needed to ascertain whether the regulation truly coincides with blastocoel formation.

Density regulatory mechanisms might be a complicating factor in comparisons of swimming rates between species. Some of the temperature sensitivity observed in embryonic swimming behavior could result from this regulatory activity. However, observations of *Strongylocentrotus franciscanus* swimming embryos indicate that, in their case, the temperature sensitivity is mediated at least in part by a change in swimming pattern. The decrease in upward velocity with increased temperature could be a useful physiological response in embryos entering a region where temperatures are higher than optimal for normal development.

#### Vertical speeds and velocity profiles

Measured sinking speeds for pre-hatching blastulae were, with one exception, significantly greater than speeds of upward swimming. This was surprising, as Mogami *et al.* (1988) found that *Hemicentrotus pulcherrimus* gastrulae swam about 3 times faster than they sank. The sinking rates reported by those authors are markedly lower than the results reported here for earlier embryonic stages. They employed a viewing chamber of 0.5-mm thickness, whereas I found measurable wall effects on sinking rates when embryos fell within 3–4 mm of a wall (Fig. 9). By restricting observations to the central 4 mm of the 19-mm chamber, wall effects were minimized. Also, these two studies define sinking embryos differently. Mogami *et al.* treated larvae with KCN to measure passive sinking rates at the same stages for which swimming rates were quantified. I have focused on slightly earlier developmental stages to achieve a similar comparison between vertical translation rates of hatched and unhatched embryos. These results demonstrate that non-swimming embryos of four echinoid species move through the water column at about the same speed as newly hatched swimmers.

If swimmers do not move more rapidly than non-swimmers, then the hypothesis that they are at a disadvantage because of increased encounters with certain types of predators (Gerritsen and Strickler, 1977; Greene, 1986) may be invalid. Effects of embryo speed on capture by predators probably vary, but studies with copepods indicated that increased prey velocity reduced the likelihood of post-



**Figure 9.** *Strongylocentrotus droebachiensis* blastulae sinking (14 °C) at various distances from the forward wall of the chamber depicted in Fig. 1. A camera lens was focused at different depths to track sinking rates and their sensitivity to wall proximity. The total inside depth of the chamber (19 mm) proved more than sufficient to allow measurement of speed of movement without the interference of wall effects. Embryos within about 4 mm of the chamber wall exhibited slowed sinking. Sinking speeds of embryos within 5 mm of the chamber center appeared stable. All sinking and swimming measurements reported in this study were taken from embryos passing within 2 mm of the chamber center.

encounter capture of ciliates whose volumes were similar to those of the embryos in this study (Jonsson and Tiselius, 1990). Rapidly sinking embryos might be as difficult for predators to capture as swimmers, though sinkers lack the helical motion and steeper velocity gradient characteristic of swimmers, with possible consequences for their vulnerability to detection and capture. Likewise, sinkers might not experience a marked disadvantage in terms of oxygen or dissolved-nutrient absorption.

The flow-field profiles of sinking and swimming *D. excentricus* blastulae demonstrate that flow characteristics differ even if actual rates of movement are more similar than previously suspected. As predicted, sinking embryos disturb the surrounding water column at a greater distance than do swimming embryos, which create steeper velocity gradients in the surrounding fluid. Natural turbulence can interfere with the ability of ambush predators to detect even short-range signals (Saiz *et al.*, 1992; Saiz and Kiorboe, 1995). Under conditions of low turbulent energy, however, ciliary activity may dampen water-column disturbance around moving embryos.

#### Swimming and oxygen stress

The steeper velocity gradient around swimming *D. excentricus* blastulae also has implications for the absorption of dissolved nutrients. Even if unhatched embryos sink at least as quickly as they are capable of swimming at hatching, the near-field velocity profile created by ciliary activity in swimming embryos could still provide swimmers with



greater nutrient and dissolved-gas flux. It is debatable whether early embryos gain anything from this flux increase. Some early embryos have been observed to transport dissolved organic molecules from the environment (Manahan, 1983; Shilling and Bosch, 1994), but the importance of such transport for embryonic growth and development has yet to be broadly established. Oxygen-sensitive phases in early urchin embryogenesis probably correspond to periods when metabolic demands are increasing. Older studies indicate that oxygen consumption in urchins increases during cleavage and plateaus briefly during the early blastula stage (summarized in Yanagisawa, 1975), whereas recent work with *Hemicentrotus pulcherrimus* shows respiratory rates that are nearly constant until hatching, after which time oxygen consumption increases continuously through gastrulation (Fujiwara *et al.*, 2000).

Rearing *D. excentricus* embryos in environments at 20%–25% and 5%–9% oxygen saturation demonstrated the impessive tolerance of these embryos for reduced-oxygen conditions. Changes in embryo size were similar across treatments, and although some delays in developmental timing resulted from low ambient oxygen, these delays were only 2–3 h. Oxygen availability was never so low that the development of hatched, swimming embryos prior to gastrulation was dramatically slowed or arrested, and size was unaffected; this suggests that swimming would rescue early planktonic embryos from hypoxia only when natural conditions were extreme.

### Conclusions

The benefits of early swimming may include ecological and physiological effects that promote embryo survivorship. The results of this study indicate several potential advantages of swimming. Measured sinking speeds were in most cases greater than upward swimming speeds, so swimming could decrease encounters with predators. The flow-field characteristics around swimming embryos were also distinct from those of sinking embryos, which may further reduce detection or successful handling by predators. Embryo swimming speed scaled negatively with size in a still-water laboratory environment, indicating a possible advantage of small size for planktonic embryos. This speed-size scaling also raises the question of turbulence effects on swimming ability. The capacity of embryos to reorient themselves after being turned is a component of swimming behavior not explored in this study, but it is likely to be as important as upward swimming rate in determining embryo progress through turbulence. The tolerance of *Dendraster excentricus* embryos for low-oxygen conditions suggests that swimming may not be crucial for ensuring oxygen delivery to developing embryos, except in unusually hypoxic environments. Finally, the unexpected results indicating density regulation in unhatched blastulae of all four species exam-

ined are intriguing and deserve closer scrutiny for both pre-swimming and swimming stages.

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